THE EFFECT OF X-RAYS ON CLEAVAGE IN ARBACIA EGGS: EVIDENCE OF NUCLEAR CONTROL OF DIVISION RATE

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There has been much speculation as to what part of the cell, after exposure to short wave-length radiation, is most responsible for bringing about certain observable biological changes—changes in rate of growth, rate of mitosis, and the like. Also, there has been much speculation as to the influence of the nucleus, if any, on the rate of cell division. The present investigation gives information on these seemingly unrelated questions.

In previous reports (Henshaw, 1932, 1933) we have described the effects of X-rays on the time of the first cleavage in the eggs of *Arbacia punctulata* when irradiated before fertilization. At this time we wish to supplement this by describing the effects when sperm alone are irradiated, and also, when both eggs and sperm are irradiated. As a background for the present work the conditions and findings of the previous work will be reviewed briefly.

Arbacia eggs can be collected in large numbers from a single female; likewise, sperm can be collected in large quantities from a single male. If arranged properly in sea water, the eggs may be fertilized with the sperm and normal development observed. If a few drops of sperm suspension are added to a sample of eggs and the time for cleavage noted, the first cleavage will be found to have occurred in half of the eggs in about 55 minutes under ordinary conditions. If these conditions are kept the same for various samples of a given collection, the cleavage time (i.e., the time from the moment of insemination to the moment when 50 per cent have cleaved) will be constant within one minute ordinarily.

If, however, the eggs are exposed to X-rays immediately before fertilization, the onset of the first cleavage is noticeably delayed. Thus, the radiation in some way affects the protoplasm of the eggs and as a result the first cellular division is slowed. If graded doses of radiation are given to a series of samples, the amount of delay will be found to increase as the dose of radiation increases. In the present study the same irradiation effect (cleavage delay) has been produced

when sperm alone were irradiated. Because sperm and eggs differ distinctly in certain respects, it has been possible to obtain some indication of what part of the cell is affected by the radiation in bringing about cleavage delay, and also something of the relation of cell parts to cell function.

Procedure and Results

Arbacia were collected during the spawning season and maintained in running sea water until needed for use. When material was required, the ovaries were removed from a single female and allowed to shed eggs into a large volume of sea water (several hundred ml.). The ovarian tissue was removed by filtering the suspension through cheesecloth and the eggs were then allowed to settle. Three drops of heavy egg suspension were placed in each of several small glass vessels and the material was then ready for treatment. The vessels had flat bottoms and were about 8 mm. in diameter. The egg suspension was about 2 to 3 mm. deep in the vessels.

Testes were removed from a single male and minced slightly with fine pointed forceps. Portions of the resulting material were then placed in other glass vessels of the same design to a depth of 2 to 3 mm. and were thus ready for treatment.

By laying out the work properly, it was possible to obtain data for three types of experiments at the same time on different portions of the same collection of material—data for (1) irradiated eggs and normal sperm; (2) irradiated eggs and irradiated sperm; and (3) normal eggs and irradiated sperm. It was necessary, therefore, to have on hand both irradiated and non-irradiated material. Accordingly, some of the vessels of sperm and eggs were not treated. The samples receiving treatment were arranged in a special way for exposure to the radiation. Since the time factor has been shown previously to have an influence on the results obtained, the same duration of exposure (30 minutes) was given to all samples. Varying doses of radiation were obtained by placing the samples at varying distances from the source of radiation.

One sample each of sperm and eggs was placed at the distances of 20, 28.3, 40 and 56.5 cm. from the center of the target of the X-ray tube. These distances were selected in accordance with the Inverse Square Law, reducing the intensity by one half in each case. Since means were not available for measuring the intensity at each distance, the relative dosages must be regarded as only approximate. This, however, does not interfere with the relative effects obtained on sperm and eggs, which is the main point of interest here. The intensity at

20 cm. distance was approximately 720 rœntgens per minute, knowledge of the exact amount not being essential. The radiation was supplied by a standard, Coolidge Type, air-cooled, tungsten target, X-ray tube. The conditions under which the radiation was obtained were 120 kv., 180 pv., 5 ma. and a filter of thin paper.

After exposure the eggs were arranged in Syracuse dishes in a larger volume of water and sperm suspension in proper dilution was

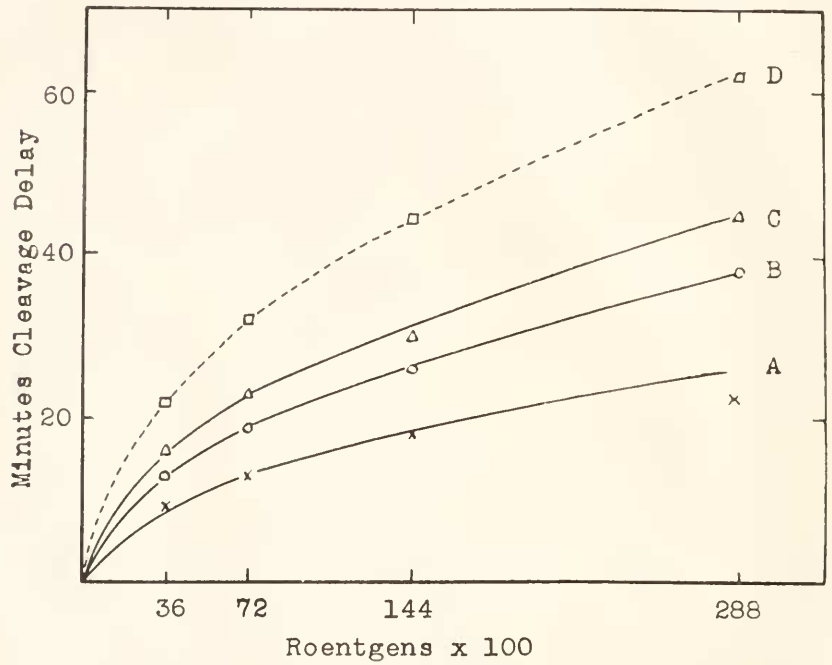

FIG. 1. Curves showing how irradiation effect (cleavage delay) varied with respect to dose of radiation administered. Curves *A* and *B* show the results obtained when eggs and sperm, respectively, were irradiated, while Curve *C* shows it when both eggs and sperm were irradiated. Curve *D* shows the effect which would have been obtained if the effects produced in sperm and eggs had been entirely additive.

added to make the combinations mentioned. Insemination of the different samples was accomplished as nearly simultaneously as possible, never more than two minutes being required for this. Determination of the cleavage time was then carried out according to the procedure described previously.

A series of nine experiments have been carried out, all of which show essentially the same thing. Averaged results for three of these, carried out under precisely the same conditions, are shown in Fig. 1.

The abscissæ indicate dose in roentgens, while the ordinates indicate irradiation effect in minutes cleavage delay. Curve *A* shows the effects obtained when eggs alone were given increasing amounts of radiation. This, in general, is like the curves obtained previously for similar conditions. Curve *B* shows the effect when sperm alone were irradiated and Curve *C* shows it when both sperm and eggs were irradiated.

It will be seen, not only that cleavage delay is produced by the irradiation of sperm alone, but also that the amount of delay is greater for a given dose when sperm are irradiated than when eggs are irradiated. While the actual amount of difference varied somewhat from experiment to experiment, the ratio of difference obtained for the two gametes remained approximately the same. Also, it will be seen that still greater effects are obtained when both eggs and sperm are irradiated. This indicates that the effects are to some extent additive when both elements are treated.

Discussion

The significance of this investigation may best be considered by analyzing step by step the processes involved. Experiments with irradiated eggs will be taken up first.

An affecting agent, radiation, was administered to a certain type of cell, the *Arbacia* egg, and changes of some kind were produced. These were made evident, at least in part, by the addition of sperm and the determination of the time required for cleavage. The sperm in such a case does two things of consequence. First, it enters the egg and incites it to division, thus initiating the reaction by which the irradiation effect is detected and measured. Second, it carries into the egg a portion of protoplasm which has not been irradiated. Hence, since radiation was allowed to affect only the egg, the procedure of adding sperm and timing cleavage may be regarded mainly as a means used in detecting irradiation effect. Changes produced in the egg, therefore, must have been responsible for the slowing of division observed.

When the same effect is obtained by irradiating sperm, quite a different problem is presented for consideration. A protoplasmic part which had previously been used to detect irradiation effect was caused to harbor changes brought about by radiation, carry them into a portion of non-irradiated protoplasm and there exert an influence which caused the untreated protoplasm to act as though it had been treated. This unique finding may be used in analyzing the action of radiation on the rate of cell division.

In the work with irradiated eggs, no information was obtained which indicated what parts of the eggs were affected by the radiation which, in turn, were responsible for the slowing of division rate. Modifications may have been produced in the membranes affecting permeability; they may have been produced in the cytoplasm causing alterations in viscosity, pH and other chemical organization; or they may have been produced in the nucleus, bringing about structural and chemical modifications. The situation, however, is different when sperm alone are irradiated.

When the normal egg is fertilized with normal sperm, the sperm penetrates the egg membranes, leaving its swimming organ at the surface. Thus, only the sperm nucleus and a small amount of cytoplasm actually enter the egg. The sperm nucleus moves forward through the egg cytoplasm to meet the egg nucleus and the two, so far as is known, share equally in the subsequent nuclear activity of the organism. In regard to the sperm cytoplasm which enters the egg, no important function has been attributed to it except one. It furnishes the functional centrosome for the first division figure, that of the egg not taking part. On entering the egg, the centrosome divides and moves to the poles of the egg, giving rise to the astral arrangement as it does so.

Thus certain points become clear. The irradiation effect which was transported to the egg by the sperm must have been carried by either the nucleus or centrosome or by both. From the fact that cleavage delay can be produced by irradiating sperm alone, it is clear that the delay does not result from alterations produced in the membranes nor in the greater bulk of cytoplasm such as that contained in the egg. Moreover, by considering the fact that the same irradiation effect is obtained when either sperm or eggs are irradiated, and the fact that the egg centrosome is not functional, the possibility of the centrosome harboring an irradiation effect which influences division rate is greatly diminished. The above results, therefore, may be cited as evidence indicating that the irradiation effect, as manifested by cleavage delay, is due to changes produced in the nucleus.

If this be true, the same evidence demonstrates in a clear manner that the nucleus exerts at least some control over the rate of cell division. X-rays have been used previously as a unique surgical tool in performing successful and effective operations on the nucleus, thereby bringing about changes in hereditary activity (Muller, 1928; 1933). The above experiments indicate that physiological activity may also be effectively modified by influencing the nucleus with this agent.

From a purely biological point of view, it is interesting to note that irradiation modification may be produced in a cell containing only half the normal number of chromosomes and that the effect becomes expressed in a cell containing the full number. Here, both an injured and a normal nucleus (haploid) are present in the same cell. It might be expected in such a case that the normal nucleus would exert a dominance over the abnormal one and thus prevent the irradiation injury from being expressed. This, on the contrary, is an example of an abnormal nucleus showing dominance over a normal one.

As yet nothing has been said in regard to the relative susceptibility of sperm and eggs to the radiation. The results, as presented, indicate that the effect is definitely greater when sperm are irradiated. If, as believed, the egg and sperm nuclei share equally in the nuclear functions of the organism, and if it is true that nuclear changes brought about by radiation are responsible for the radiation effect observed, it would be expected that equal effects would be produced on sperm and eggs by equal doses of radiation. The differences observed, however, are appreciable and tend to indicate a differential response on the part of the gametes. But there are reasons for believing that the differences observed are not real.

First, Heilbrunn and Young (1935) in a recent paper have reported work done on the same material and with the same source of radiation. In brief, they irradiated eggs in sea water in one case and in ovarian tissue in another and found noticably more cleavage delay when ovarian tissue was present during treatment. They suggest that this is evidence indicating the presence of *necrohormones*—an irradiation product which is produced in one tissue and which is diffused out and influences another. As a matter of convenience in our experiments, the eggs were irradiated in sea water and the sperm were irradiated in the presence of testicular material. Should necrohormones have been produced by this material, they would have caused the irradiation effect to be greater than it otherwise would have been. Second, the discrepency may be accounted for by differences in absorption in the two cases. The eggs settled to the bottom of the vessel during treatment and were protected by two to three millimeters of sea water, while at least part of the sperm were held near to the exposed surface by the testicular material. Since unfiltered radiation was used, two millimeters of sea water probably removed an appreciable amount of otherwise effective radiation. These factors, however, have not been investigated, thus making it impossible to discuss their significance at the present time.

Perhaps the most interesting phase of this investigation lies in the

set of facts implied by the quantitative results. The reaction in its simplest form appears to be one of indirect dependence. That is, the effect observed (cleavage delay) is dependent on the amount of radiochemical change of a particular kind which is produced in the living system and which in turn is dependent on the amount of radiation administered. How the observed effect varies with respect to the quantity of radiation given is indicated by the shape of the curves obtained, but how the radiochemical change varies with respect to the dose of radiation applied and how the observed effect varies with respect to the amount of radiochemical change, is less obvious. The data, however, may be analyzed to obtain certain indicative information on these points.

Since it has been possible to produce the same irradiation effect by exposing either gamete, it is probable that the same radiochemical change (that which causes cleavage delay) is produced in the sperm and eggs indifferently. Through fertilization, then, two quantities of radiochemical change, known to produce definite amounts of cleavage delay, are brought together in the same system where their combined effects are expressed as one. This unique procedure, made possible only by the fact that cleavage delay may be produced by irradiating either gamete, offers an indirect method of investigating the quantitative aspects of the radiochemical change involved. For example, if the combined effect observed is equal to the sum of the two effects obtained independently, it follows that the observed effect is directly proportional to the amount of radiochemical change whatever it may be. Curve *D* in Fig. 1 shows the combined effects obtained by adding Curve *A* to Curve *B*.

The curve actually obtained when both elements were irradiated falls considerably below Curve *D*, indicating that the relation is not directly proportional. However, Curves *C* and *D* appear to be similar in shape, which indicates that the observed effect is equal to the combined calculated effect times some constant less than one. This has been found to be very nearly 0.72 at various points along the curves in the figure. Insofar as these results are trustworthy, this indicates that a linear relation exists between the radiochemical change produced and the biological effect observed. Since, however, the quantitative data now available are limited, it seems best not to carry the analysis farther at this time nor to emphasize the relationships observed. At this time it is sufficient to point out that a new method is available for investigating certain phases of the radiobiological reactions about which very little is known.

Summary

1. Experiments have been carried out to investigate the influence of X-rays on the division rate of *Arbacia* eggs.

2. It was found, first, that the time required for the first cleavage was noticably prolonged when the eggs were irradiated before fertilization.

3. Second, it was found that the same effect could be produced by irradiating the sperm before they were allowed to fertilize normal eggs. Thus, an irradiation effect was carried by one gamete into another where the effect became expressed. The non-irradiated gamete was, therefore, caused to behave as though it had been irradiated.

4. Irradiation of both eggs and sperm indicated that the effect obtained was to some extent additive.

5. It was pointed out that only the nucleus and centrosome of the sperm appear to have an important influence on the egg and that the irradiation effect in such a case must have been carried by one or both of these. Reasons were given for believing that the centrosome was not important in this respect, thus indicating that the slowing of cell division observed was due to irradiation effects produced in the nucleus.

6. It appears, therefore, that the nuclei of the cells studied exert at least some control over the rate of cell division, and it is significant that such control is manifested when the damage is produced in a haploid nucleus and the effect is expressed in the presence of a normal haploid nucleus.

7. A method is presented for investigating quantitatively some of the more remote aspects of the radiobiological reactions.

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